PATENT COOPERATION TREATY

the
INTERNATIONAL SEARCHING AUTHORITY

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PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* 14 March 2005 (14-03-2005)

Applicant's or agent's file reference 000595-0052	**FOR FURTHER ACTION** See paragraph 2 below	
International application No. PCT/CA2004/001968	International filing date *(day/month/year)* 15 November 2004 (15-11-2004)	Priority date *(day/month/year)* 13 November 2003 (13-11-2003)
International Patent Classification (IPC) or both national classification and IPC IPC[7]: A61K 35/74; A61K 38/00; A61K 35/00		
Applicant BIO-K PLUS INTERNATIONAL INC. ET AL		

1. This opinion contains indications relating to the following items :

[X]	Box No. I	Basis of the opinion
[ ]	Box No. II	Priority
[X]	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
[X]	Box No. IV	Lack of unity of invention
[X]	Box No. V	Reasoned statement under Rule 43*bis*.1(a)(I) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement.
[ ]	Box No. VI	Certain documents cited
[X]	Box No. VII	Certain defects in the international application
[X]	Box No. VIII	Certain observations on the international application

2. **FURTHER ACTION**
If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/CA *Canadian Intellectual Property Office* *Place du Portage I, C114 - 1st Floor, Box PCT* *50 Victoria Street* *Gatineau, Quebec K1A 0C9* Facsimile No: 001(819)953-2476	Authorized officer Cynthia Brewer (819) 997-4921

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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language which it was filed, unless otherwise indicated under this item.

   [ ] This opinion has been established on the basis of a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of :

   a. type of material

      [ ] a sequence listing

      [ ] table(s) related to the sequence listing

   b. format of material

      [ ] in written format

      [ ] in computer readable form

   c. time of filing/furnishing

      [ ] contained in the international application as filed.

      [ ] filed together with the international application in computer readable form.

      [ ] furnished subsequently to this Authority for the purposes of search.

3. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statement that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments :

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of :

[ ] the entire international application

[X] claim Nos. 19 to 23

because:

[X] the said international application, or the said claim Nos. 19 to 23 relate to the following subject matter which does not require an international preliminary examination *(specify)* :

Although claims 19 to 23 are directed to methods of treatment of the human/animal body which this Authority is not required to examine under Rule 67.1 (iv) of the PCT, the written opinion has been established on the basis of the alleged effects of the compositions or supernatants referred to therein.

[ ] the description, claims or drawings *(indicate particular elements below)* or said claim Nos. _____ are so unclear that no meaningful opinion could be formed *(specify)* :

[ ] the claims, or said claim Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

[ ] no international search report has been established for said claim Nos. _____.

[ ] the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that :

the written form [ ] has not been furnished

[ ] does not comply with the standard

the computer readable form [ ] has not been furnished

[ ] does not comply with the standard

[ ] the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

[ ] See Supplemental Box for further details.

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Box No. IV Lack of unity of invention

1. [ ] In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has :

   [ ] paid additional fees

   [ ] paid additional fees under protest

   [ ] not paid additional fees

2. [ ] This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   [X] complied with

   [ ] not complied with for the following reasons :

4. Consequently, this opinion has been established in respect of the following parts of the international application :

   [X] all parts

   [ ] the parts relating to claim Nos. _____

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Box No. V **Reasoned statement under Rule 43*bis*.1(a)(I) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	27 to 30	YES
	Claims	1 to 26, and 31	NO
Inventive step (IS)	Claims	None	YES
	Claims	1 to 31	NO
Industrial applicability (IA)	Claims	1 to 31	YES
	Claims	None	NO

2. Citations and explanations :

Reference is made to the following documents:
D1: WO 03/045405 A2 (LUQUET, F-M. et al.) 5 June 2003
D2: ARIMOCHI, H. et al. BIOCHEM BIOPHYS RES COMMUN. 1997, Vol. 238, No.3, pages 753-757.
D3: KATO, I. et al. LIFE SCI. 1998, Vol. 63, No.8, pages 635-644.
D4: MITAL, B.K. et al. CRIT REV MICROBIOL. 1995, Vol.21, No.3, pages 175-214.
D5: MATSUZAKI, T. INT J FOOD MICROBIOL. 1998, Vol.41, No.2, pages 133-140.
D6: GRIFFIOEN, A.W. et al. PHARMACOL REV. 2000, Vol.52, No.2, pages 237-268.

Novelty and Inventive Step - Articles 33(2) and 33(3) PCT
The problem to be solved by the instant application is the provision of a nutraceutical product that has antiangiogenic properties to be used in the prevention or treatment of an angiogenesis dependent disorder, for example, cancer. The disclosed nutraceutical formulations are lactic compositions comprising bacterial strains *Lactobacillus acidophilus* and *Lactobacillus casei* either alone or in combination. Alternatively, the lactic compositions are cell-free supernatants derived from the conditioned media of the *Lactobacillus* cultures. The present application discloses that the cell-free supernatants inhibit formation of capillary structures *in vitro* and inhibit the VEGF-induced migration of endothelial cells *in vitro*.

Document D1 discloses lactic compositions comprising bacterial strains *Lactobacillus acidophilus*, including strain I-1492, and *Lactobacillus casei* either alone or in combination. In addition, the disclosed lactic compositions are useful in the prevention or treatment of an angiogenesis dependent disorder, for instance, tumor growth. Further, it is noted that recitation of "angiogenesis dependant disorder" or "antiangiogenic agent" are non-distinctive characteristics and encompass the uses disclosed in D1. Further, document D1 discloses cell-free supernatants derived from the bacterial cell cultures. It is noted that document D1 does not disclose the characterization of any antiangiogenic properties inherent in said supernatants. However, further characterization of the biological properties of an old and known product does not reinstate novelty to said product. Consequently, D1 is considered to be prejudicial to the novelty of claims 1 to 8, 14 to 26, and 31 which are thus, not compliant with Article 33(2) PCT.

Document D2 discloses the use of an animal model to demonstrate the *in vivo* inhibition of aberrant crypt foci formation, i.e. precursor lesions of colon cancer, by cultures of *Lactobacillus acidophilus* as well as supernatants derived therefrom. Further, the culture supernatants were obtained by centrifugation of *Lactobacillus acidophilus* cultures and then sterilized by filtration through a filter with a pore size of 0.45 µm (see page 754, left col). In addition, the observed inhibition of carcinogenesis was attributed to unidentified substances within the supernatant derived from the *Lactobacillus acidophilus* cultures. Thus, in view of D2 there is no doubt that *Lactobacillus* cultures as well as supernatants derived therefrom are useful in the prevention or treatment of an angiogenesis dependent disorder, namely, cancer. Therefore, inclusion of the characteristics "antiangiogenic agent", "antiangiogenic properties", or "angiogenesis dependent disorder" do not set forth technical features of the instantly claimed products and uses thereof which can distinguish them from the disclosure of D2. Accordingly, claims 1, 5 to 10, 12 to 15, 17 to 19, 21 to 24, and 26 are considered to be anticipated by D2 and therefore are not compliant with Article 33(2) PCT.

Continued: see Supplemental Box

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted :

Contrary to the requirements of Rule 5.1 (a) (ii) PCT, relevant background art is not cited in the description.

The following inconsistencies in the description should also be considered:

page 1, line 9, the word "Description" contains a typo; and

page 16, line 34, and page 17, line 3, the text of the description does not correspond to the figure number indicated.

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 1 to 4 do not meet the requirements of Article 6 PCT. Inclusion of "is useful" in claim 1 lacks clarity. Consequently, it is not clear if applicant's intent is to claim a lactic composition per se or to claim a specified use of a lactic composition. Clarification is thus required.

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of : Box V, 2. Citations and Explanation

Document D3 discloses the ability of *Lactobacillus casei* to reduce the incidence and development of type II collagen-induced arthritis in mice which is an animal model of rheumatoid arthritis in humans. The suppressive effects were attributed to modulation of humoral and cellular immune responses by *Lactobacillus casei,* for example, a reduced production of interferon-gamma. Thus, D3 discloses the ability of a lactic composition to treat and prevent an angiogenesis disorder, namely, rheumatoid arthritis. Consequently, document D3 is considered novelty destroying for claims 1, 14, 15, 17, 19, 21, and 23 under Article 33(2) PCT.

Even if applicant were to overcome the above novelty objections based on the distinction of an antiangiogenic effect, the following analysis with regard to inventive step of claims 1 to 23 would apply:

Document D4 is a review article which discloses the health benefits associated with consumption of lactic compositions containing *Lactobacillus acidophilus* and summarizes numerous *in vitro* and *in vivo* studies. In particular, document D4 recapitulates the known anticarcinogenic, hypocholesterolemic and antagonistic activities against intestinal pathogens of *Lactobacillus acidophilus.*

Document D5 is a review article which discloses the anti-tumour, anti-metastatic and immunomodulatory activity of *Lactobacillus casei* both *in vitro* and *in vivo*.

Document D6 is a review article dedicated to the subject of angiogenesis. In addition to the molecular pathways associated with physiological angiogenesis document D6 reviews aspects of pathological angiogenesis as a target to treat or prevent diseases associated with excessive angiogenesis, such as, cancer and chronic inflammation e.g. rheumatoid arthritis and psoriasis.

None of the cited prior art documents explicitly disclose the use of a lactic composition comprising *Lactobacillus acidophilus* and/or *casei* or a supernatant derived therefrom as an antiangiogenic agent. However, in view of document D2 taken with either document D4 or D5 in combination with document D6 an inventive step is not acknowledged under Article 33(3) PCT for the subject matter set forth in claims 1 to 23.

Firstly, it is well established in the art, for instance, reviewed in either document D4 or D5 that lactic compositions comprising *Lactobacillus* possess anti-tumour, anti-metastatic and immunomodulatory characteristics. Secondly, document D2 discloses that *Lactobacillus* culture supernatants contain unidentified inhibitory substances. Moreover, these biological effects have been demonstrated using a variety of *in vivo* animal models. In contrast, the alleged invention discloses *in vitro* data which looked at end-points with respect to formation of capillary structures by HUVECs and migration assays in response to VEGF.

The prior art acknowledges that the precise molecular mechanisms, by which *Lactobacillus* compositions and/or their supernatants (i.e. conditioned media) exert these known beneficial effects, have not been fully elucidated. Nevertheless, it is well established that *Lactobacillus* compositions and/or their supernatants are useful in the prevention and treatment of disorders which are associated with pathological angiogenesis, for instance, cancer and rheumatoid arthritis. In particular, a skilled person would appreciate anti-metastatic activity is correlated with the ability to act as an antiangiogenic agent, for example, see document D6. Further, applicant has not demonstrated any improvement, advantage or unexpected effect of the instantly claimed uses. Accordingly, the ability of a lactic composition comprising *Lactobacillus* or a supernatant derived therefrom to act as an antiangiogenic agent does not appear to impart any technical characteristic which could be used to distinguish the claimed products and uses from those disclosed in documents D2, D4 or D5 which pertain to the treatment and prevention of an angiogenesis dependent disorder.

Claims 27 to 30 lack an inventive step under PCT Article 33(3) as being obvious in view of either D1 or D2 and the common general knowledge in the art. Notably, both D1 and D2 disclose a bacterial culture supernatant obtained via centrifugation. Moreover, methods of obtaining cell culture supernatants is considered to fall within the scope of routine laboratory practice. Thus, the additional features characterizing alternative method steps in claims 27 to 30 are all known to the skilled person and these method steps are obvious alternatives in a cell fractionation method.

Industrial Applicability - Article 33(4) PCT

Claims 1 to 18, and 24 to 31 appear to define subject matter that has industrial applicability under Article 33(4) of the PCT, based on the function of lactic compositions or supernatants derived therefrom of the instant application.

For the assessment of claims 19 to 23 on the question of whether or not they define subject matter that has industrial applicability, no unified criteria exists in the PCT. Further the patentability of said claims can depend on their formulation. Although the methods *per se* defined in claims 19 to 23 relate to subject matter which this Authority is not obliged to examine under Rule 67.1 (iv) of the PCT, the use of lactic compositions or supernatants derived therefrom referred to therein for the prevention or treatmer of an angiogenesis dependent disorder appears to represent subject matter that has industrial applicability.